

SUPPLEMENTARY MATERIALS FOR

Viruses in Vietnamese patients presenting with community acquired sepsis of unknown cause

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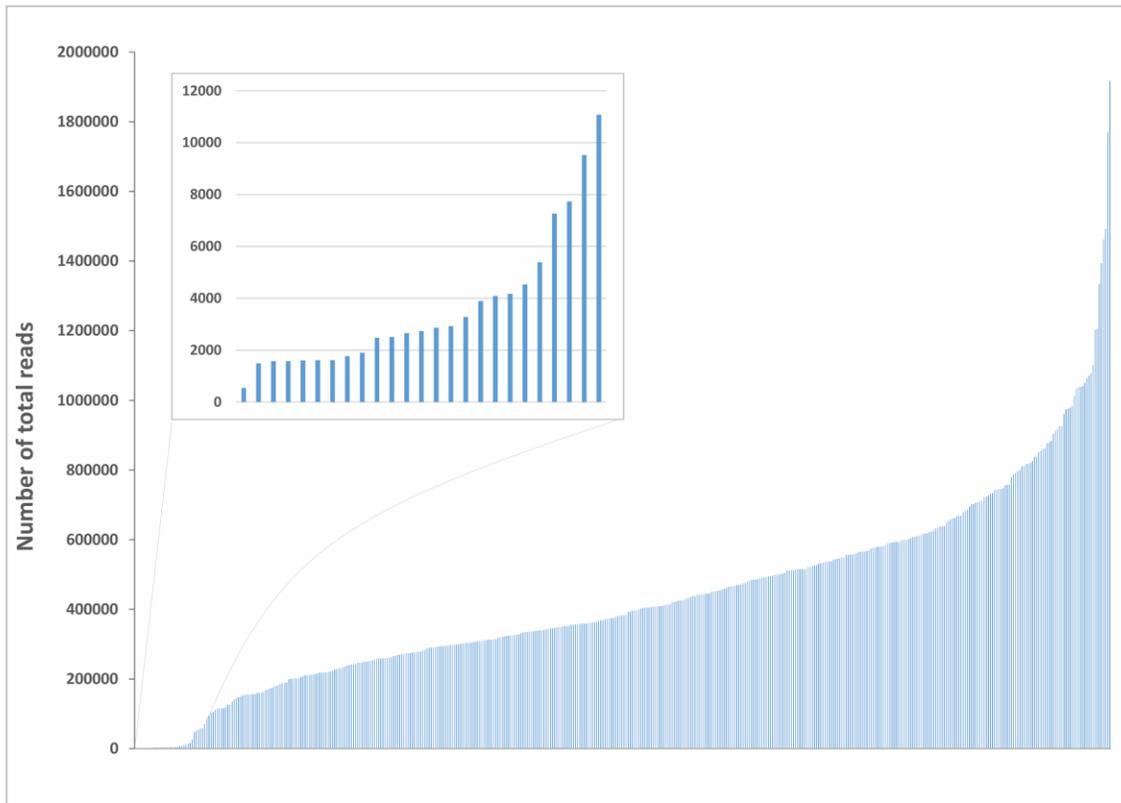
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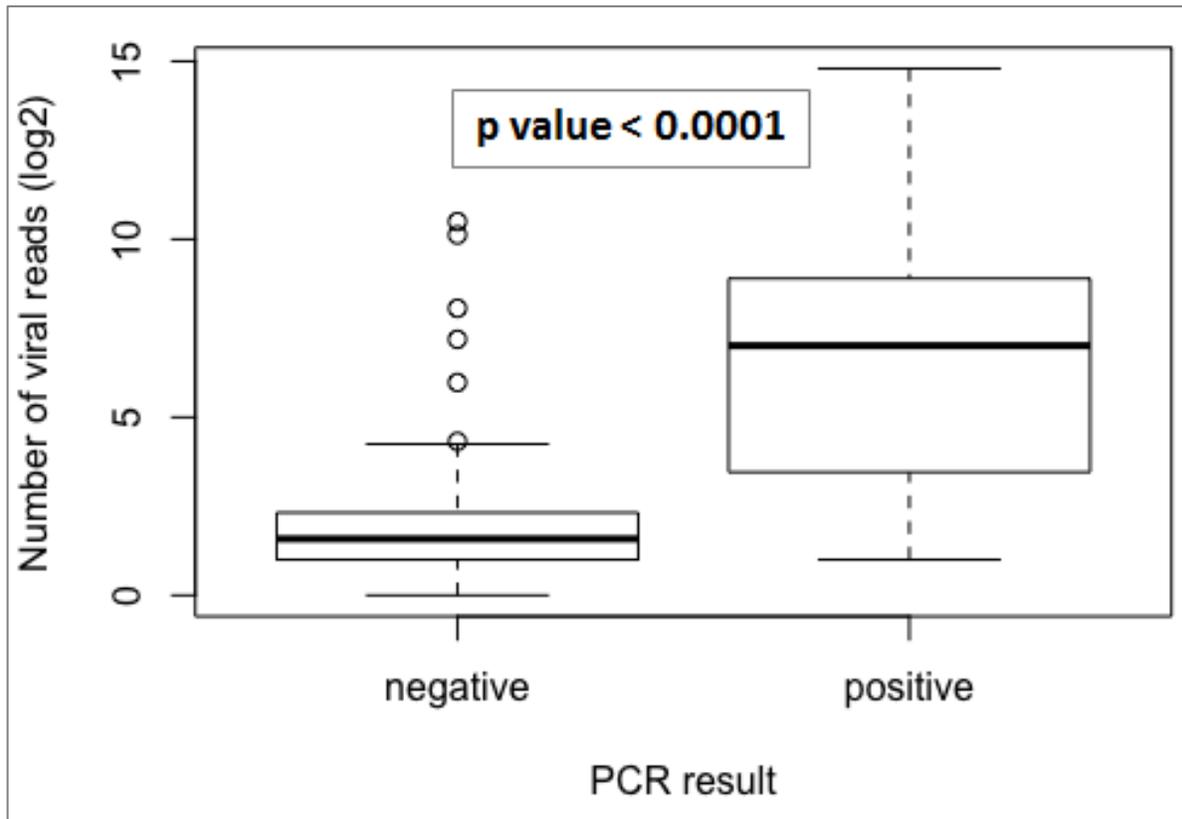
Keywords: community acquired sepsis, viral metagenomics, Vietnam

Running title: Metagenomic NGS of sepsis patients of unknown cause

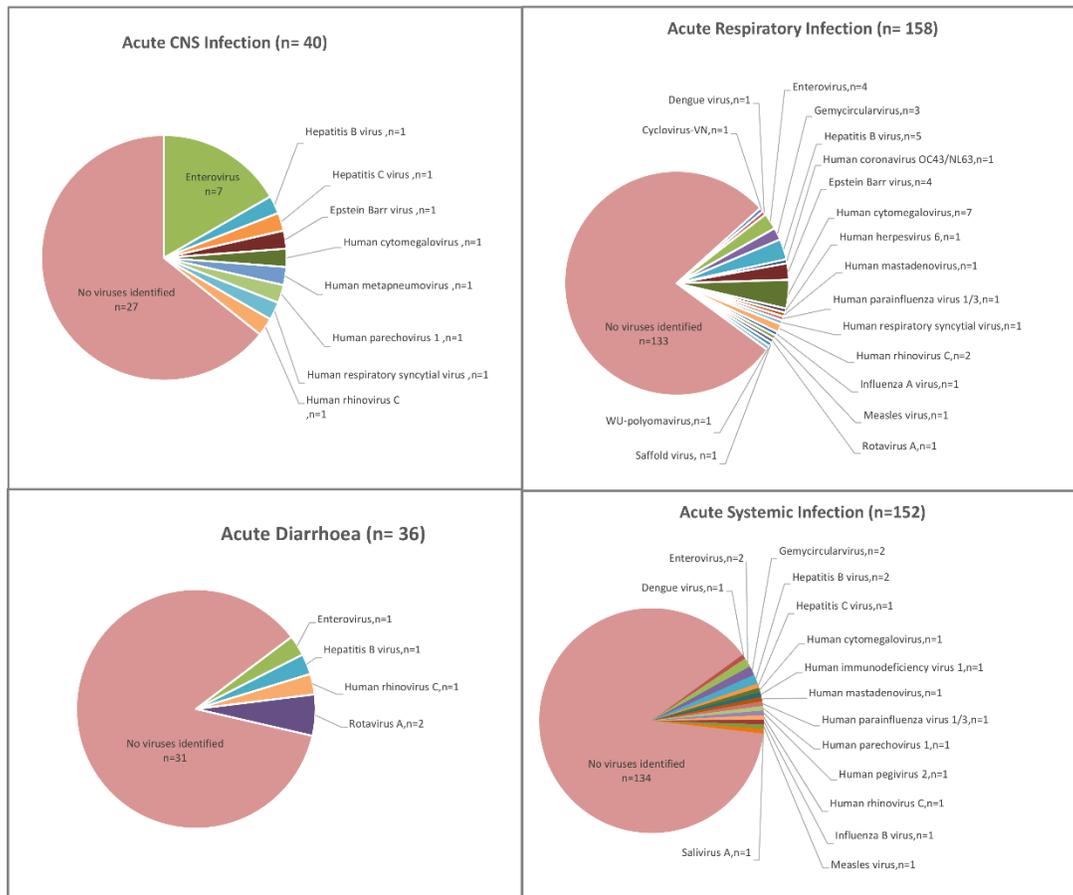
Supplementary Figure 1: Bar chart showing the number of reads obtained from individual samples. Each vertical bar represents one sample.



Supplementary Figure 2: Boxplots showing the difference in the numbers of viral hits between PCR positive and negative groups

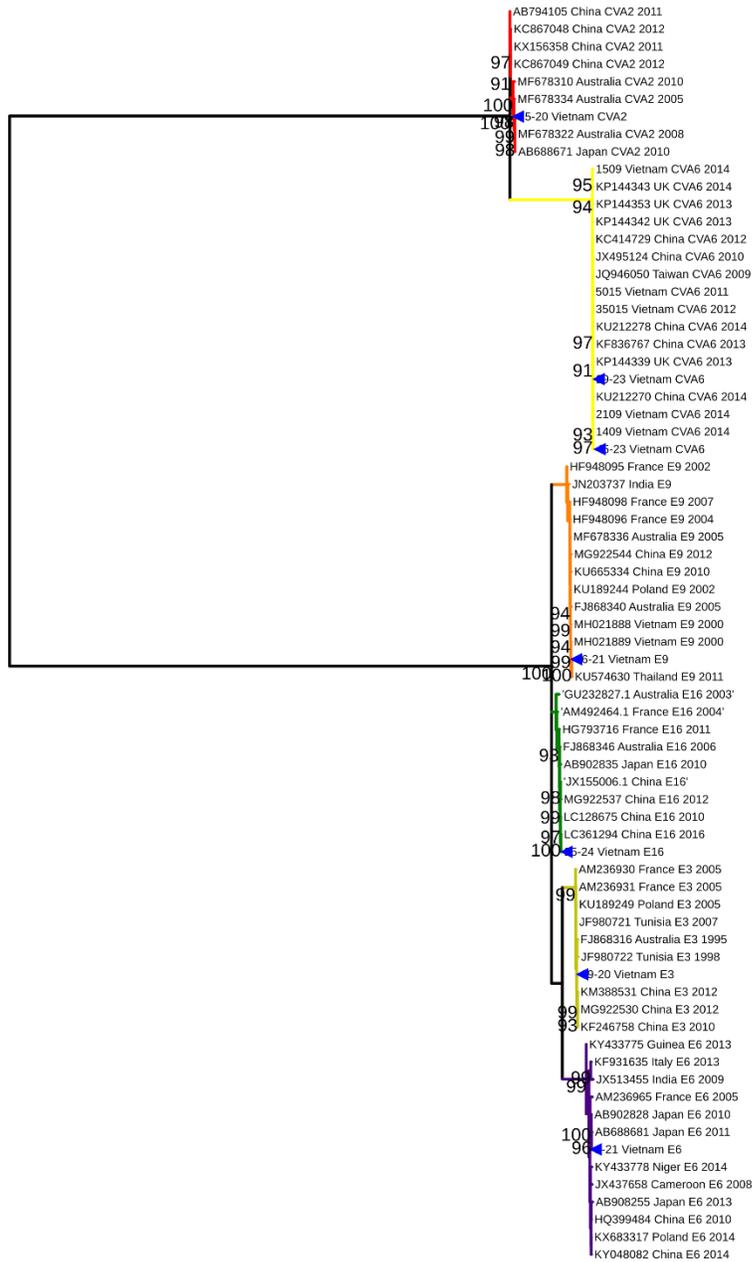
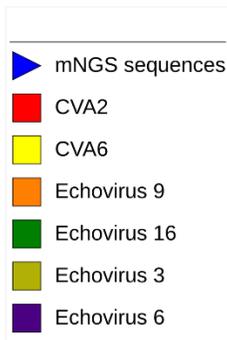


Supplementary Figure 3: Viral detection by mNGS, which were then confirmed by viral specific PCR, in different clinical entities

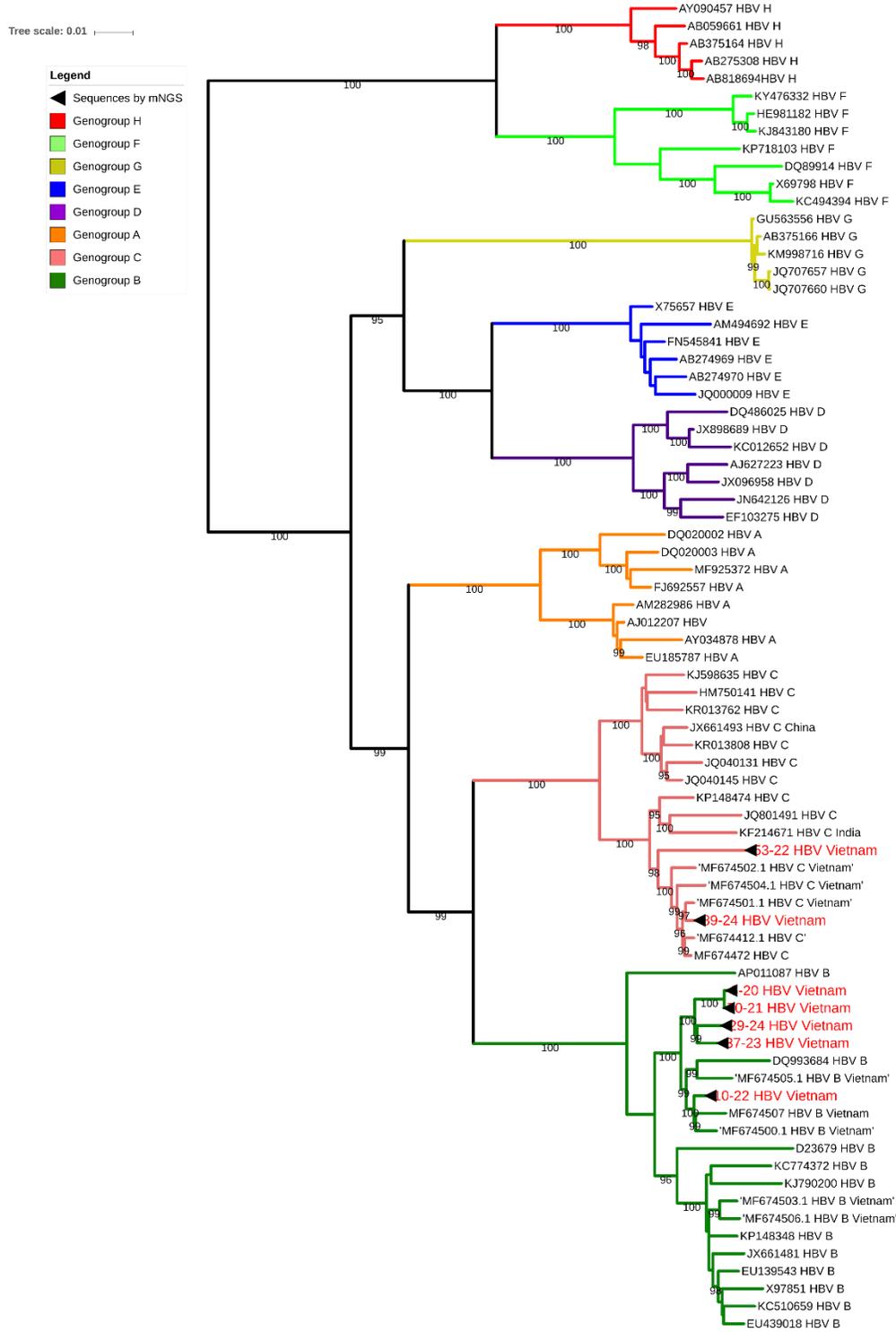


Supplementary Figure 4: Maximum Likelihood tree based on complete VP1 sequences of different enterovirus serotypes illustrating the relatedness between enterovirus serotypes recovered in the present study by mNGS (blue triangles) and representative EV serotypes

Tree scale: 10



Supplementary Figure 5: Complete coding sequence based Maximum Likelihood tree showing the relationship between HBV sequences recovered in the present study (black triangles) and representative HBV genotypes



Supplementary Table 1: Diagnostic work-up carried out as per the study protocol of the original report

Pathogens ^{#, **}	Assay #1 and samples	Assay #2 and samples	References
Leptospirosis	Whole-blood PCR	Microagglutination tests of paired sera	[1, 2]
Scrub typhus	Whole-blood PCR	IFA of paired sera	[3, 4]
Rickettsiosis and murine typhus	Whole-blood PCR		[4, 5]
Murine typhus	IFA of paired sera		[3]
Bacteraemia	Whole-blood PCR		[6, 7]
Hantavirus	Serum PCR		[8]
Japanese encephalitis virus	CSF ELISA		[9]
Mumps	CSF ELISA		Mumps virus (Parotitis) IgM ELISA Kit (IBL International, Germany)
Measles	CSF ELISA		Measles virus IgM micro-capture ELISA (IBL International, Hamburg, Germany)
Rubella	CSF ELISA		Rubella virus IgM micro-capture ELISA (Novatec Immundiagnostica Technologie & Waldpark, Germany)
Dengue	CSF PCR		[10]
Herpes simplex virus 1 and 2	CSF PCR		[11]
Varicella-zoster virus	CSF PCR		[12]
Enterovirus	CSF PCR		[13]
<i>Parechovirus</i>	CSF PCR		[14]
<i>N. meningitidis</i>	CSF PCR		[15]
<i>S. pneumoniae</i>	CSF PCR		[15]

<i>H. influenza</i> type b	CSF PCR		[15]
<i>S. suis</i>	CSF PCR		[16]
Respiratory viruses	Pooled nasal-throat swab PCR		[14]
Respiratory bacteria	Pooled nasal-throat swab PCR		[17–19]
Adenovirus	Stool PCR		[20]
Astrovirus	Stool PCR		[21]
Norovirus	Stool PCR		[22]
Rotavirus	Stool PCR		[22]

Notes to Supplementary Table 1:

#Diagnostic tests performed in every case as part of standard of care at participating hospitals including complete blood count, blood culture, urine culture, gram/ZN smears, and sputum culture if patients have respiratory symptoms, stool examination and stool culture if patients have diarrheal symptoms and CSF examination and CSF culture if patients have neurological symptoms and CNS infection is suspected.

**Diagnostic tests performed in every case per study protocol including dengue RDT (NS1 and IgM, Standard Diagnostics, South Korea), influenza RDT (QuickVue, Quidel Corporation, USA), only for paediatric patients age < 7 years old and leptospirosis RDT (Leptospira IgM/IgG, Standard Diagnostics), only for paediatric patients age ≥ 7 years old and all adult patients

*Four multiplex real-time PCR assays detecting 15 virus subtypes of 10 viruses; Influenza (A & B), Adenovirus, Enterovirus, Respiratory syncytial virus (A & B), Metapneumovirus, Rhinovirus, Parainfluenza virus (1, 2, 3 & 4), Coronavirus, Bocavirus (subtype OC43 & NL63), and Parechovirus

§5 real-time assays detecting 5 bacteria; *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydomphila pneumoniae*, *Chlamydomphila psittaci* and *Bordetella pertussis*

Supplementary Table 2: List of primers and probes used for subsequent PCR confirmation experiments

Viruses	Oligo sequence (5'-3')			Sources
	Forward	Reverse	Probe	
Measles	ATTACATCAGGATCCGG	GTATTGGTCCGCCTCATC		[23]
HBV	GGACCCCTGCTCGTGTACA	GAGAGAAGTCCACCMCGAGTCTAGA	FAM-TGTTGACAARAATCCTCACAATACCRGAGA-TAMRA	Newly designed
Rotavirus	ACC ATC TWC ACR TRA CCC TC	GGT CAC ATA ACG CCC CTA TA	FAM-ATG AGC ACA ATA GTT AAA AGC TAA CAC TGT CAA-BHQ1	[24]
Enterovirus	CCCTGAATGCGGCTAAT	ATTGTCACCATAAGCAGCC	CY5-ACCCAAAGTAGTCGGTCCG -BHQ3	[25]
Dengue	AAGGACTAGAGGTTAGAGGAGACCC	CGTTCGTGCCTGGAATGATG	FAM- AACAGCATATTGACGCTGGGAGAGACCAGA-BHQ1	[26]
Dengue 2	CCATACACGCCAACATGAA	GGGATTTCCTCCCATGATTCC	FAM-AGGGTGTGGATTTCGAGAAAACCCATGG-BHQ1	[27]
HIV1	GGTGCGAGAGCGTC	ATGCTRTCATCATYTCTTC		[28]
	ATGGGTRAARGTARTAGAAGAAAAGGG	CTGCCTGRTGYCCYCCCACTA		
HCV	AGACTGCTAGCCGAGTAGYGTGG	TGCTCATGDTGCACGGTCTACGA	FAM-TTGTGGTACTGCCTGATAGGGTGCTT -BHQ1	Newly designed
PIV 1	ATCTCATTATTACCYGGACCAAGTCTACT	CATCCTTGAGTGATTAAGTTTGATGATA	CYAN500-AGGATGTGTTAGAYTACCTTCATTATCAATTGGTGATG-DB	[29]
PIV2	CTGCAGCTATGAGTAATC	TGATCGAGCATCTGGAAT	LCRED610-AGCCATGCATTACCAGAAGCCAGC-BBQ	[29]
PIV3	ACTCTATCYACTCTCAGACC	TGGGATCTCTGAGGATAC	LCRED670-AAGGGACCACGCGCTCCTTTCATC-BBQ	[29]
PIV4	GATCCACAGCAAAGATTAC	GCCTGTAAAGAAAGCAGAGA	HEX-TATCATCATCTGCCAAATCGGCAA-BHQ1	[29]
Coronavirus OC43	GGTGGYGGGAYGATATGTTACG	KRTTGGCATAGCACGATCACA	6-FAM-ATGTTGACAAYCCTGTWCTTATGGGTTGGG-MGBNFQ	[29]
Coronavirus NL63	GCTRAGCATGATTTCTTTACTTGG	CARTYTKTKTCATCAAAGTTACGCA	6-FAM-CAGARTCAATTATGGTAATGTTAGTAGACA-MGBNFQ	[29]
PEV	GGGTGGCAGATGGCGTGCCATAA	CCTRCGGGTACCTTCTGGGCATCC		[30]
	YCACACAGCCATCCTCTAGTAAG	GTGGGCCTTACAACACTAGTGTTTG		
Rhinovirus	AGSCTGCGTGGCKGCC	ACACGGACACCCAAAGTAGT	CYAN500-TCCTCCGGCCCTGAATGYGGCTAAAYC-DB	[29]
MPV	AGCTTCAGTCAATTCAACAGAAG	CCTGCAGATGTGCGCATGT	LCRED670-TGTTGTGCGGCAGTTTTTCAGACAATGC-BBQ	[29]
FA	GACAAGACCAATCCTGTCACYTCTG	AAGCGTCTACGCTGCAGTCC	LCRED610-TTCAGCTCACCGTCCAGTGAGC-BBQ	[29]
FB	TCGCTGTTTGAGACACAAT	TTCTTTCCACCGAACCA	CYAN500-AGAAGATGGAGAAGGCAAAGCAGAACT-DB	[29]
RSV	ATGAACAGTTTAACATTACCAAGT	GTTTTGCCATAGCATGACAC	LCRED610-TGACTTCAAAAACAGATGTAAGCAGCTCC-BBQ	[29]
			LCRED610-TTATGACATCAAAAACAGACATAAGCAGCTCAG-BBQ	
ADV	CAGGACGCTCGGRGTAYCTSAG	GGAGCCACVGTGGGRTT	LCRED670-CGGGTCTGGTGAGTTTGCCCGC-BBQ	[29]
Saffold virus	CTAATCAGAGGAAAGTCAGCAT	GACCACTTGGTTGGAGAAGCT		[31]
	CAGCATTTCGGCCAGGCTAA	GCTATTGTGAGGTCGCTACAGCTGT		
Salivirus	CCCTGCAACCATTACGCTTA	CACACCAACCTTACCCACC		[32]
	ATTGAGTGGTGCAYGTTG	ACAAGCCGGAAGACGACTAC		Newly designed

Wu-polyomavirus	TGTTACAAATAGCTGCAGGTCAA	GCTGCATAATGGGGAGTACC		[33]
Human herpesvirus 6	TTTGCAGTCATCACGATCGG	AGAGCGACAAATTGGAGGTTTC		[34]
Human herpesvirus 4	GAGGAATTGCCCTTGCTATT	CCTTAGTGGGCCAGGTTGT	FAM -TCGTCTCCCCTTTGGAATGGC-TAMRA	Newly designed
Human herpesvirus 5	CCAAGCGGCCTCTGATAACCA	GGTCATCCACACTAGGAGAGCAGA	FAM-ATGAAGCGCCGATTGAGGAGATCT-TAMRA	[35]
Gemycircularvirus	GTGGTAATGGTCGTCGGTATTC	CCTCATCATTTCGTAGTAAGCAATCTC A		[36]
	AGTCCTGAATGTTTCCACTCG	CAAGCGTTCCTCGAAAATGAC		Newly designed
Cyclovirus VN	GAGCGCACATTGAAAGAGCTAAA	TCTCCTCCTCAATGACAGAAACAAC	FAM-CGADAATAAGGMATACTGCTCTAAAGSTGGCG-BHQ1	[37]
Human pegivirus 2	CGCTGATCGTGCAAAGGGATG	GCTCCACGGACGTCACACTGG	CY5-GCACCCTCCGTACAGCCTGAT-BHQ2	[38]

Supplementary Table 3: Detection of ≥ 2 viruses in the same samples/patients

Detected in	Serum	Pooled swabs	Stool
1 Adult	HBV		HBV and Measles virus
1 Child		Enterovirus, Influenza A and Cytomegalovirus	ND
1 Child	Cyclovirus VN and Gemycircularvirus	Cytomegalovirus	ND
1 Child		Enterovirus and Human rhinovirus A	ND
1 Child		Enterovirus and Human Herpesvirus 4	ND
1 Child	Enterovirus	Cytomegalovirus and Human herpesvirus 4	ND
1 Adult	Human immunodeficiency virus, Hepatitis C virus and Human Pegivirus 2	ND	ND
1 Adult	HBV and Dengue	ND	ND
1 Adult	ND	ND	Measles and Salivirus A
1 Child	ND	Cytomegalovirus and Human respiratory syncytial virus	ND
1 Child	ND	Cytomegalovirus and Human mastadenovirus	ND
1 Child	ND	Human herpesvirus 6 and Saffold virus	ND
1 Child	ND	Enterovirus and Human metapneumovirus	ND

Note to Supplementary Table 6: ND: not detected

Supplementary Table 4: List of common contaminants and viruses not reported in human samples

Viral family	Species	Genome	Number of matching reads	Best BLASTx E value	Detected in (n)	Other virus found	Patient group	Related species was previously reported in	References
<i>Adenoviridae</i>	Bovine mastadenovirus C	dsDNA	4	3.47E-12	Serum (1)		Adults	Cattle	[39]
<i>Coronaviridae</i>	Bulbul coronavirus HKU11	ssRNA	2	2.92E-06	Serum (1)		Adults	Wild bird	[40]
<i>Coronaviridae</i>	Penaeus monodon circovirus VN11	ssRNA	2,5&15	9.46E-05	Pooled swabs (n=1) Serum (2)		Children and Adults	Numerous including pigs	[41]
<i>Nodaviridae</i>	Nodamura virus	ssRNA	2	9.13E-11	Stool (1)	Shuangao insect virus 11	Adults	Insects	[42]
<i>Picornaviridae</i>	Boone cardiovirus	ssRNA	17	5.45E-07	Serum (1)		Children	Rats	[43]
<i>Picobirnaviridae</i>	Dromedary picobirnavirus	dsRNA	8	4.90E-87	Pooled swabs (1)		Adults	Camels	[44]
<i>Parvoviridae</i>	Bat parvovirus	ssDNA	3	1.22E-08	Serum (2)		Children	Bat	[45]
<i>Papillomaviridae</i>	Human papillomavirus	circular dsDNA	4	2.86217E-42	Serum (1)		Adult		
<i>Parvoviridae</i>	Densovirus	ssDNA	≤ 559	5.31243E-96	Pooled swabs (12) Serum (38) CSF (1)		Children and Adults	Mosquitoes	[46]
<i>Partitiviridae</i>	Partitivirus	dsRNA	≤ 152	2.0824e-103	Pooled swabs (4) Serum (22)		Children and Adults	Fungi	[47]
<i>Parvoviridae</i>	Parvovirus NIH-CQV	ssDNA	≤ 104	2.43E-52	Pooled swabs (4) Serum (87) Stool (1) CSF (3)		Children and Adults	Qiagen column contaminant	[48]
<i>Reoviridae</i>	Kadipiro virus	dsRNA	3	7.87E-32	Serum (5)		Adults and Children	Contaminant	[49, 50]

<i>Reoviridae</i>	Lutzomyia reovirus 1	dsRNA	24	5.54E-07	Serum (1)		Adults	Sand flies	[51]
<i>Reoviridae</i>	Eubenangee virus	dsRNA	1	9.23E-05	Serum (1)	Tilligerry virus	Adults	Marsupials, cattle, mosquitoes and <i>Culicoides</i>	[52]
<i>Reoviridae</i>	Cypovirus	dsRNA	1,4,7	4.03196E-40	Serum (3)		Children and Adults	Insect	[53]
<i>Rhabdoviridae</i>	Curionopolis virus	ssRNA	6	4.45E-05	Serum (1)		Children	Culicoides	[54]
<i>Totiviridae</i>	Saccharomyces cerevisiae virus L-BC (La)	dsRNA	9	7.45478E-21	Pooled swabs (1) Serum (3)		Children and Adults	Fungi	[55]
<i>Totiviridae</i>	Scheffersomyces segobiensis virus L	dsRNA	≤ 857	3.50723E-118	Pooled swabs (3) Serum (12) Stool (2) CSF (1)		Children and Adults	Fungi	[56]
Unclassified	Magnaporthe oryzae RNA virus	RNA	9,14,15&21	1.06135E-39	Serum (4)		Children and Adults	Fungi	[57]
Unclassified	Mosquito VEM virus SDRBAJ	ssDNA	3	1.40E-07	Serum (1)	CRESS virus	Adults	red snapper tissue	Unpublished paper
Unclassified	Nepavirus	ssDNA	2	6.44E-06	Serum (1)		Children	Untreated Sewage	[58]

Supplementary Table 5: Viral species found in (different body compartments of) adult patients with a SOFA score of ≥ 2

Patients	Sera	Pooled nasal and throat swabs	Stool
1	Rotavirus A	ND	ND
2	Hepatitis B virus	ND	Measles and hepatitis B virus
3	Dengue	ND	ND
4	Gemycircularvirus	ND	ND
5	ND	Epstein-Barr virus	ND
6	ND	ND	Measles and Salivirus A

Note to Supplementary Table 5: ND: not detected

Supplementary Table 6: The frequency of six most common viruses detected by mNGS in different clinical entities

Clinical presentation	Enterovirus (N=14)	Hepatitis B virus (N=9)	Cytomegalovirus (N=9)	Rhinovirus (N=5)	Epstein-Barr virus (N=5)	Rotavirus (N=3)
CNS infection, n (%)	7 (50)	1(11.1)	1(11.1)	1(20)	1(20)	0
Respiratory infection, n (%)	4 (28.6)	5(55.6)	6(66.7)	2(40)	4(80)	0
Diarrhea, n (%)	1(7.1)	1(11.1)	0	1(20)	0	3(100)
Systematic infection, n (%)	2(14.3)	2(22.2)	2(22.2)	1(20)	0	0

Supplementary Table 7: The number of viral reads and genome coverage in individual samples

Virus	Sample type	Number of total reads	Number of viral reads	E-value	Percentage of genome coverage (contig length/genomic size, bp)
Cyclovirus VN	Serum	442730	17	1.43937E-53	90.0% (1,671/1,856)
Cytomegalovirus	Swabs	493794	9	2.51E-38	>1% (624/235,403)
Cytomegalovirus	Swabs	211254	25	4.4231E-66	>1% (1,046/235,272)
Cytomegalovirus	Swabs	504418	200	1.4765E-109	1.3% (2,861/223,782)
Cytomegalovirus	Swabs	591732	12	2.90191E-52	>1% (900/235717)
Cytomegalovirus	Swabs	855702	4	5.94419E-25	>1% (309/235,272)
Cytomegalovirus	Swabs	1101990	1629	0	20.1% (44,960/223,782)
Cytomegalovirus	Swabs	1203206	53	3.20843E-51	1.9% (4,517/236,032)
Cytomegalovirus	Swabs	533616	86	8.87413E-53	2.2% (5,402/235,834)
Cytomegalovirus	Swabs	126026	204	4.7457E-111	7.1% (15,891/223,782)
Dengue virus	Serum	427286	3828	0	94.9% (10,174/10,723)
Dengue virus	Serum	560500	4721	0	95.0% (10,188/10,723)
Enterovirus	Serum	565748	405	0	42.0% (3,077/7,328)
Enterovirus	Serum	861012	6536	8.5729E-57	97.8% (6,795/6,946), 26.4% (1,941/7,345)
Enterovirus	Serum	348940	22	9.1753E-53	27.9% (1,841/6,606)
Enterovirus	Swabs	711594	329	0	31.4% (2,318/7,345)
Enterovirus	Swabs	493794	11	8.23215E-53	8% (579/7,345bp)
Enterovirus	Swabs	876786	29	1.5706E-155	61.8% (4,390/7,104)
Enterovirus	Serum	443284	170	5.827E-180	45.5% (3,379/7,434)
Enterovirus	Swabs	904408	6	2.31739E-22	2% (146/7,206)
Enterovirus	Swabs	825274	787	0	48.5% (3,204/6,612)
Enterovirus	Serum	172824	131	1.5905E-169	19.1% (1,421/7,432)
Enterovirus	Serum	200880	14	7.2996E-36	7.2% (537/7,427)
Enterovirus	Serum	349020	166	0	13.2% (980/7,433)
Enterovirus	Serum	205366	184	0	10.7% (703/6,591)
Enterovirus	Swabs	102766	5	1.30753E-39	2% (127/7,368)
Epstein-Barr virus	Serum	755526	4	2.59398E-36	<1% (336/169,864)
Epstein-Barr virus	Swabs	604874	3	1.98845E-32	1.6% (2,789/169,864)
Epstein-Barr virus	Swabs	904408	2	4.13442E-11	1.6% (2,795/169,864)
Epstein-Barr virus	Swabs	732950	2	1.18234E-12	1.5% (2,607/169,864)
Epstein-Barr virus	Swabs	126026	6	3.45686E-24	1.7% (2,905/169,864)
Gemycircularvirus SL1	Serum	381902	1668	3.06932E-60	100% (2,199)
Gemycircularvirus SL1	Swabs	883776	23	1.4508E-112	77.2% (1,697/2,199)
Gemycircularvirus SL1	Serum	442730	41	1.49764E-60	3.8% (85/2,199)
Gemycircularvirus SL1	Serum	281200	11	1.649E-102	52.5% (1,156/2,199)
Gemycircularvirus SL1	Swabs	219956	2	1.79015E-49	22.5% (494/2,199)
Hepatitis B virus	Serum	11076	183	5.56485E-51	94.7% (3,044/3,215)
Hepatitis B virus	Stool	441248	127	1.074E-142	78.7% (2,529/3,215)
Hepatitis B virus	Serum	560500	2	2.66806E-54	9.3% (299/3,215)
Hepatitis B virus	Serum	649082	2	1.08203E-30	5% (175/3,215)
Hepatitis B virus	Serum	298130	22918	7.56657E-56	100% (3,215)
Hepatitis B virus	Serum	352212	982	6.7019E-158	95.6% (3,074/3,215)
Hepatitis B virus	Serum	438146	18364	3.42494E-56	100% (3,215)

Hepatitis B virus	Serum	54750	1732	5.6804E-124	100% (3,215)
Hepatitis B virus	Serum	374364	477	6.3359E-179	90.6% (2,914/3,215)
Hepatitis B virus	Serum	320784	75	0	84% (2,709/3,215)
Hepatitis C virus	Serum	293316	5342	1.40256E-56	98.6% (9,171/9,297)
Hepatitis C virus	Serum	231960	307	2.06237E-50	74.6% (6,984/9,358)
Human coronavirus	Swabs	1203206	4	1.86221E-28	1.1% (328/30,521)
Human herpesvirus 6	Swabs	1050246	16	8.72727E-53	0.8% (1,309/161,296)
Human immunodeficiency virus	Serum	293316	355	8.4618E-102	55.1% (4,883/8,860)
Human mastadenovirus	Swabs	855702	6	5.02331E-30	1.6% (582/35,831)
Human mastadenovirus	Swabs	975450	287	0	36.2% (12,774/35,265)
Human metapneumovirus	Swabs	825274	522	0	74.5% (9,932/13,327)
Human parainfluenza virus	Swabs	701436	3	8.16848E-51	2.5% (393/15,502)
Human parainfluenza virus	Swabs	111152	427	0	59.0% (9,047/15,335)
Human parechovirus	Serum	331722	58	0	15.8% (1,155/7,320)
Human parechovirus	Stool	608352	52	7.29444E-96	16.2% (1,186/7,320)
Human pegivirus 2	Serum	293316	273	0	33.9% (3,237/9,538)
Human respiratory syncytial virus	Swabs	504418	28422	0	99.3% (15,165/15,276)
Human respiratory syncytial virus	Swabs	452112	9	3.04048E-35	3% (467/15,232)
Human rhinovirus	Serum	513280	483	0	59.4% (4,217/7,099)
Human rhinovirus	Swabs	811032	401	0	14.6% (974/6,692bp)
Human rhinovirus	Swabs	408734	8	3.66242E-55	5.4% (387/7,208bp)
Human rhinovirus	Swabs	489110	39	5.99188E-55	25.0% (1,761/7,047)
Human rhinovirus	Swabs	876786	67	0	61.8% (4,390/7,104)
Influenza A virus	Swabs	493794	23	3.39944E-53	5.2% (710/13,500)
Influenza B virus	Swabs	479434	594	6.7272E-145	58.4% (1,025/1,755)
Measles virus	Stool	441248	19530	0	96.6% (15,360/15,894)
Measles virus	Stool	435356	4	2.60588E-49	3.8% (602/15,894)
Rotavirus A	Serum	960504	2	5.84582E-25	<1% (155/18,550)
Rotavirus A	Serum	590870	2	1.59659E-12	<1% (100/18,550)
Rotavirus A	Serum	491942	366	0	83.0% (2,731/3,292)
Saffold virus	Swabs	1050246	29	4.5541E-138	9.2% (737/8,054)
Salivirus A	Stool	435356	4	1.95271E-37	7.3% (582/8,021)
WU Polyomavirus	Swabs	459132	164	1.1621E-129	45.3% (2,367/5,229)

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